MICROBIAL OXIDATION AND REDUCTION OF SULFUR IN COALS FOR SPECIATION AND DESULFURIZATION

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1. INTRODUCTION

The microbial removal of sulfur from coal by oxidative means is well documented, especially with regard to pyritic sulfur removal by organisms such as *Thiobacillus ferooxidans* and *Sulfolobus acidocaldarius*. There also have been reports of the removal of organic sulfur from coals, (1,2) although generally less success has been achieved along these lines. Two aspects of microbial coal interaction which have received less attention are the use of microbes to elucidate coal sulfur chemistry and the removal of coal sulfur by bio-reduction. It is unlikely that there are significant amounts of elemental sulfur in pristine coal samples (3) but the recent work of Narayan suggests the presence of elemental and/or polysulfide sulfur in some weathered coals (4). By ASTM techniques these sulfur forms would be counted as "organic" sulfur. The lack of a direct assay for organic sulfur makes it difficult to establish a clear connection between microbial metabolism and the cleaving of covalently bound sulfur from the coal matrix. It also ignores any chemical interconversion of sulfur species which may occur during weathering, desulfurization, or analytical processing.

In this study we have made use of organisms which selectively metabolize elemental sulfur, either oxidizing it to sulfate at 28°C (*Thiobacillus thiooxidans*) or reducing it to hydrogen sulfide at 98°C (*Pyrodictium brockii* and *Pyrococcus furiosus*). We have pursued both the development of a bioassay for the speciation of sulfur in coals and novel desulfurization methods. The quantification of elemental sulfur by bioassay was confirmed with CS_2 extraction of the coals. Narayan has suggested that a significant fraction of so called organic sulfur in coals may be amenable to transformation to free elemental sulfur under mildly oxidative conditions. We will attempt to oxidize several coals using such techniques to investigate the possibilities for novel routes to microbial "organic" sulfur removal. The activity of the organisms toward polysulfide sulfurs (amorphous), partially oxidized species, and other model compounds (i.e. dibenzothiophene) will also be investigated.

The reduction of sulfur to hydrogen sulfide may avoid one of the problems involved with oxidative coal desulfurization, namely, the re-deposition of insoluble sulfate bearing minerals such as jarosite from the leachate when sulfate concentrations become too high. We are also investigating the activity of cell free extracts and lysed membrane preparations towards coal desulfurization.

1.1. The Microorganisms

Thiobacillus thiooxidans is an acidophilic, aerobic, sulfur-oxidizing chemolithotrophic bacterium. Strain 8085 was obtained from the American Type Culture Collection, Rockville, MD. *Pyrodictium brockii*, an autotroph, and *Pyrococcus furiosus*, a heterotroph, are both hyperthermophilic, anaerobic, sulfur-reducing archaebacteria, capable of growth above 100°C (5,6,7). *P. brockii* strain DSM 2708 and *P. furiosus* strain DSM 3638 were both obtained from the Deutsche Sammlung von Mikroorganismen, Gottingen, Federal Republic of Germany.

2. EXPERIMENTAL METHODS

2.1. Coals

Samples of four different coals were used in this study. Indiana no. 5 (IBCSP sample no. 6) and Illinois no. 6 (IBCSP sample no. 1) coals were obtained from C.W. Cruse, Director of the Illinois Basin Coal Sample Program. Victorian brown coal (Australia) was obtained from D.J. Allardice, Coal Corp. of Victoria, through L. Atherton of the Electric Power Research Institute. A waste coal from Indiana (bog coal) was obtained from R. Narayan, Purdue University.

2.2. Coal Biodesulfurization Experiments

2.2.1. Thiobacillus thiooxidans. Coals to be used in these experiments (200-400 mesh) were sterilized by heating in a vacuum oven at 85°C for one hour on each of three successive days. Coal (0.5 to 1.5 g) was aseptically added to 50 ml of the sterile low sulfate medium (minus elemental sulfur) in 250 ml conical flasks. The flasks were inoculated with an active culture of *T. thiooxidans* previously grown in the low sulfate medium containing elemental sulfur. Coals were tested in duplicate or triplicate flasks. Sterile controls and flasks containing cells and no coal were run with each experiment. Flasks were incubated at 28°C with shaking at 200 r.p.m. After sulfate concentrations leveled off (generally after one to two weeks), the difference in solution sulfate concentrations between inoculated and uninoculated flasks was determined. From this value the amount of elemental sulfur converted to sulfate was calculated.

2.3.2. Pyrodictium brockii and Pyrococcus furiosus. Coals to be tested (200-400 mesh) were washed with reverse osmosis water and then dried in an air oven at 70°C for about four hours. Coals were tested in the range of 0.2 to 2.0 % pulp density (w/v). Bottles were incubated quiescently at 98°C. Gas phase H_2S was assayed by syringe injections to a Varian 3700 gas chromatograph using a thermal conductivity detector, a 6-foot, packed Hayesep-N column, and a carrier gas flow rate of 30 cc/min (helium). The total amount of sulfide generated was estimated using Henry's Law. Cell densities were determined by direct count of periodically taken subsamples. The cells were stained with acridine orange and visualized on 0.2 μm filters using epifluorescence microscopy.

2.3. Chemical Determinations

Sulfate ion in solution was determined turbidimetrically using barium chloride precipitation (8). Total sulfur content in coals was determined using a modified high temperature method (9) employing a Leco (St. Joseph MI) furnace and automatic titrator. The method is based on an ASTM method for sulfur in petroleum products (10). NBS coal standard reference materials were used for system calibration. Forms of sulfur in coals (sulfatic, pyritic, organic) were determined using ASTM procedures (11). Elemental sulfur was determined chemically by extracting 0.5 g of coal with 25 ml of CS_2 at room temperature for at least 12 hr. The coal was removed by filtration and the CS_2 was evaporated to dryness. The residue was taken up in hexane and assayed for sulfur colorimetrically (12).

3. RESULTS

3.1. Sulfur Oxidation by *Thiobacillus thiooxidans*

The amount of sulfur in various forms in the test coals is shown in Table 1. Especially noteworthy is the very low level of pyritic sulfur in the Australian coal and the very high level of total and sulfatic sulfur in the bog coal. This latter coal is evidently very weathered.

Small amounts of sulfate above control values were produced when *T. thiooxidans* was incubated with Indiana and Illinois coal samples indicating low levels of elemental sulfur in these coals. No significant differences in sulfate concentrations were found between inoculated and uninoculated flasks containing the Australian brown coal. However, significant sulfate levels above control values were seen with the Indiana bog coal. Calculations of the elemental sulfur content in these coals based on sulfate production are shown in Table 2. Very little elemental sulfur was detected by bioassay in the first three coals, corresponding with the results of CS_2 extraction. About 1.2% (weight) sulfur as elemental sulfur was detected by bioassay in the bog coal. The total sulfur content in the bioprocessed bog coal was about 1% lower than sterile controls. This corresponded closely to the loss in "organic sulfur" from the coal (Table 2). By ASTM definitions, the organisms removed about 25% of the "organic sulfur" from the coal.

3.2. Sulfur Reduction by *Pyrodictium brockii*

Table 3 shows estimates of elemental sulfur content of the bog coal by bioassay. Liquid phase sulfide concentrations were estimated assuming the system was at equilibrium at the end of the experiment and using Henry's law to calculate dissolved sulfide concentrations. Since the pH of the sample was typically about 5.5, it was assumed that all dissolved sulfide was molecular hydrogen sulfide. The inoculated samples containing the bog coal typically yielded gas phase sulfide concentrations 4 to 5 times those obtained from uninoculated controls and inoculated samples containing no coal. Samples of other coals used in this study showed no biotic sulfide generation although bacteria could be grown in these samples if spiked with elemental sulfur (data not shown). It is interesting to note that the drop in ASTM organic sulfur levels of about 30-40% correspond well with the elemental sulfur estimate by bioassay.

3.3. Sulfur Reduction by *Pyrococcus furiosus*

Table 4 shows the activity of *P. furiosus* on the coal samples used in this study. Note that for pulp densities of 0.5% (weight), growth was strongest for the bog coal. In fact, significant amounts of sulfide production were noted only in that sample.

As can be seen, the level of sulfide production and cell yields are proportional to the amount of coal added to the medium. The three replicates at the 2% (weight) pulp density show that reproducibility in these experiments is good. The uninoculated with coal and inoculated without coal control bottles had only trace amounts of H_2S. These results strongly suggest that coal sulfur was used as a substrate by the cells.

Not reported are bioassay estimates of elemental sulfur in the bog coal. Unlike *P. brockii* which grows at a pH of 5.5, *P. furiosus* grows at a pH of about 7.0. Because sulfide levels in the liquid phase were not measured directly, estimates of dissolved sulfide levels at pH 7 or higher are more difficult to make. The liquid phase dissociation of molecular hydrogen sulfide must be accounted for. Nonetheless, initial estimates indicated that elemental sulfur in the bog coal based on sulfur reduction by *P. furiosus* was between 1% and 2% by weight. Direct solution sulfide analysis will improve these estimates.

4. DISCUSSION

Several reports of microbial removal of organic sulfur from coal have appeared in the literature. However, the definition of organic sulfur is not precise or universal. By ASTM methods, organic sulfur represents all sulfur that is not pyritic or sulfatic. If significant elemental sulfur occurs in coal or is produced during studies on coal biodesulfurization, the apparent success of organic sulfur removal will depend on the analytical scheme. By ASTM designations all three organisms used in this study removed significant (25-40%) amounts of the "organic" sulfur present in the bog coal. At the same time, in the other three coals, very little elemental sulfur was detected by bioassay or CS_2 extraction. In these coals, a loss of ASTM organic sulfur following bioprocessing (which we did not detect) would be interpreted as a loss of true organic (C-S) sulfur. It is important, therefore, that investigators determine if elemental sulfur is present in their test coals, or perhaps more important, if it forms during experimentation as a result of biotic and/or abiotic reactions.

A more complicated situation arises in consideration of data presented by Narayan et al. (4) suggesting the presence of polysulfides in coal which oxidize on weathering to produce free elemental sulfur. We have not yet determined with model compounds whether or not it is likely that *T. thiooxidans*, *P. brockii* or *P. furiosus* (or other microorganisms) can attack such polysulfide linkages directly. Experiments are needed to determine if oxidative treatment of coals leads to the production of elemental sulfur and if polysulfides are attacked by these or other microorganisms. If such forms of sulfur are important in coals, there are very good prospects for their removal. However, the organisms which would remove this type of organic sulfur will be different from the organisms which would remove, for example, thiophenic sulfur.

Ultimately, definitions do not matter if the sulfur can be removed from coal. However, the choice of organisms and engineering designs will be quite different depending on the forms of sulfur that the organisms and their enzymes encounter.

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Table 1. Sulfur in Test Coals by ASTM Procedures (%)

	Total S	Sulfate S	Pyritic S	Organic S
Illinois No. 6	4.25	0.08	0.73	3.44
Indiana No. 5	3.91	0.12	1.57	2.22
Australian	4.35	0.63	0.03	3.69
Bog Coal	11.6	5.20	2.32	4.06

Organic S = Total S - Sulfate S - Pyritic S
(ASTM Method 5.05)

Table 2. Elemental Sulfur in Coals - Thiobacillus thiooxidans

			Total Sulfur Determinations (%)		
Coal Type	Total S (%)	S° (%) (bioassay)	After CS_2 extraction	After Thiobacillus thiooxidans	sterile
Ill. #6	4.25	0.02	4.29	3.97	3.84
Ind. #5	3.91	0.03	3.76	3.39	3.49
Aust.	4.35	trace	4.50	nt	nt
Ind. Bog	11.6	1.19	8.37	5.26	6.22

nt - not tested

Table 3. Pyrodictium brockii on Bog Coal

	Total S (wt %)	Sulfatic S (wt %)	Pyritic S (wt %)	Organic S (wt %)	S° (wt %) (bioassay)
sterile	5.97	0.09	1.82	4.06*	
+ cells	4.22	0.14	1.81	2.27	1.62
(Low Inoc.)				$\Delta = 1.79$	
sterile	5.97	0.09	1.82	4.06**	
+ cells	4.98	0.15	1.96	2.87	1.40
(High Inoc.)				$\Delta = 1.19$	

apparent organic sulfur removal: * 44.1% and ** 29.3% respectively

Table 4. Activity of Pyrococcus furiosus on Coal Samples

Coal Type	Pulp Density	Cell Growth	Gas phase H_2S Production (μmoles)
Illinois No. 6	5.0	+	trace
Indiana No. 5	5.0	+	trace
Australian	5.0	+	trace
	0.0	+	trace
Bog Coal	5.0	+	43.2
Bog Coal	10.0	++	72.4
Bog Coal	20.0	uninoc.	trace
Bog Coal	20.0	+++	104.4
Bog Coal	20.0	+++	100.8
Bog Coal	20.0	+++	108.9